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Comparison of gavage, water bottle, and a high-moisture diet bolus as dosing methods for quantitative D-xylose administration to B6D2F1 (*Mus musculus*) mice

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Summary

Gavage, water bottle, and diet incorporation are 3 dosing methods used orally to administer test compounds to rodents. These 3 methods were compared in mice to determine which represented the most quantitative delivery system. For dietary incorporation, a high-moisture bolus form of NIH-31 rodent meal was developed using hydroxypropyl methylcellulose as an autoclave-stable binding agent. A high-moisture bolus was selected to increase the acceptability of the dosed diet and to promote quantitative consumption through reduced wastage. The test compound used was D-xylose, a pentose sugar that may be quantitatively detected, colorimetrically, in urine following oral dosing. Six male and 6 female B6D2F1 mice were placed in metabolism cages and dosed with a known quantity of D-xylose by each of the 3 methods. Urine was collected before and after each method of administration and analysed for total D-xylose; the per cent recovery was based upon the amount of D-xylose consumed. Quantitative consumption was apparently greatest for water bottle dosing with an average recovery of 56·0% of the original D-xylose dose. High-moisture bolus incorporation ranked second with 50·0% D-xylose recovery, and gavage was third with 41·0% D-xylose recovery.

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A major concern in dosing rodents with test compounds is the accurate quantification of oral delivery. The water bottle delivery method continuously supplies water-soluble compounds over a period of time. Problems limiting the usefulness of water bottles include test compound palatability and solubility, spillage (Lang *et al.*, 1984), soundness of the bottle stopper, leaching of compounds from the stopper (Kennedy & Beal, 1988), and individual variations in water demand (Weisburger & Weisburger, 1967). Gavage delivers a known quantity of test compound in a single dose. The disadvantages of gavage dosing include oesophageal or stomach damage, intubation of the lungs, and large, potentially fatal spikes in test compound plasma concentration (Weisburger & Weisburger, 1967; Lindamood *et al.*, 1988). The gavage dosing of animals is also very labour-intensive.

High-moisture diets contain a binder that combines water and the diet meal into a semi-solid mixture. This mixture is a useful method for presenting dusty, volatile, or toxic test compounds to animals with minimal spillage and wastage, thus reducing the risk of exposure to toxic test compounds to the technical staff (Lang *et al.*, 1984; Clapp & Bradbrook, 1982). If the test compound is pre-mixed into a soluble diet ingredient, such as lipophilic compounds mixed

with the fat component, water-soluble or -insoluble compounds can be incorporated into the diet (Weisburger & Weisburger, 1967). Studies using high-moisture, semi-purified diets containing agar as a binder have been successfully used to feed mice (Lang *et al.*, 1984), rats (Clapp & Bradbrook, 1982), and guineapigs (Navia & Lopez, 1973). Recent studies show agar induces non-pathological, physiological changes in caecum and colon cell growth; however, it may promote the carcinogenicity of certain compounds (Shiau & Wang, 1988). Compared with gavage, dosed diets have been used to deliver higher levels of an unpalatable and highly toxic compound with a reduced mortality rate (Lindamood *et al.*, 1988).

The objectives of this study were to develop a high-moisture, natural-ingredient dietary form useful for efficiently providing test compounds in a pre-feeding bolus, and to compare it with gavage and water bottle methods for efficiency in oral delivery of a known quantity of D-xylose to B6D2F1 mice. The bolus was formulated with a commercial food binder and tested for acceptability by male and female B6D2F1 mice (*Mus musculus*). D-Xylose, a pentose sugar, was chosen as a test compound based on its water solubility, rapid urinary clearance (Craig & Atkinson, 1988), lack of toxic effects, ease of detection (Eberts *et al.*, 1979), and because its absorption from the intestinal tract is proportional to the dose given (Stradley *et al.*, 1986).

Materials and methods

High-moisture diet formulation

Eight food-processing companies supplied 20 commercial food binders for this study. The classes

Table 1. Commercial food binders evaluated

Product name	*Binding agent*	*Selected binder*	*Commercial source*[a]
H-50	Cassava starch	+	1
IF-131	Cassava starch	–	1
National 78-1272	Cassava starch	–	1
Redisol 412	Cassava starch	–	2
Redisol 248	Potato starch	–	2
Thin-n-Thik 99	Corn starch	–	2
Sta-Mist 365	Corn starch	–	2
Sta-Mist 454	Corn starch	–	2
Methocel (MC)	Methylcellulose	–	3
Methocel (HPMC)	Hydroxypropyl methylcellulose	+	3
CMC R-75-H4	Sodium carboxymethylcellulose	+	4
CMC R-95-H4	Sodium carboxymethylcellulose	–	4
Avicel PH-101	Microcrystalline cellulose	–	5
Avicel RC-591F	Microcrystalline cellulose	–	5
Rhodigel	Xanthan gum	–	6
Polydextrose	Dextrose polymer	–	7
1701 Dextrin	Corn dextrin	–	8
Lo-Dex 10	Corn malto-dextrin	–	8
1620 Dextrin	Corn dextrin	–	8
1710 Dextrin	Corn dextrin	–	8

[a]Binders were selected based upon selection criterion pre-established for this research; selection is not a test of product endorsement.
[1]National Starch and Chemical Corp., Bridgewater, NJ.
[2]A. E. Staley Manufacturing, Decatur, IL.
[3]Dow Chemical, Midland, MI.
[4]Louisiana Chemical Polymers, Baton Rouge, LA.
[5]FMC Corporation, Philadelphia, PA.
[6]Rhône-Poulenc, Monmouth Junction, NJ.
[7]Pfizer Chemical Division, New York, NY.
[8]American Maize-Products, Hammond, IN.
+ Acceptable or – non-acceptable by test criteria.

of binders supplied were as follows: chemically-modified and unmodified tapioca starches, modified and unmodified corn starches, modified potato starch, hydroxypropyl methylcellulose, methylcellulose, microcrystalline cellulose, sodium carboxymethylcellulose, xanthan gum, polydextrose, malto-dextrin, and modified dextrins (Table 1).

Autoclave stability of the binders and their usefulness in high-moisture diet applications were determined by practical tests. Various combinations and varying proportions of binder, diet and water were autoclaved to determine physical stability and qualitative properties of the diet bolus formed. Binders were first autoclaved individually to determine their ability to retain pre-autoclaving form and consistency. Binders exhibiting physical changes or deterioration after autoclaving were eliminated from further consideration (Table 1).

Binders exhibiting stable properties were then autoclaved with ground (20 mesh) NIH-31 standard rodent diet (Purina Mills Inc., Richmond, IN) in dry premixes containing 1, 5, or 10% binder, by weight. The NIH-31 diet was chosen as it remains physically stable when autoclaved and is a natural-ingredient, completely balanced diet. Animals were maintained on pelleted NIH-31 diet when not on test. Dry premixes that did not undergo physical changes or deterioration were then hydrated with 5 ml aliquots of water, added incrementally, and hand-mixed until a bolus was formed. If a cohesive bolus did not form when a total of 15 ml had been added to the dry premix, no further water was added.

Mixtures of binder, diet, and water were then formulated for autoclaving and evaluation. Mixtures contained 1, 5, or 10% of binder by weight. Water, as 33, 50, or 60% of the total weight, was added to the dry ingredients prior to autoclaving, resulting in 9 samples tested per binder. Binders to be tested for animal acceptability represented 3 chemical classes and were superior in autoclave stability and cohesive properties. The 3 binders selected were Methocel® hydroxypropyl methylcellulose (Dow Chemical USA, Midland, MI), CMC R-75-H4 carboxymethylcellulose (Louisiana Chemical Polymers, Baton Rouge, LA) and H-50 modified cassava starch (National Starch & Chemical Corp., Bridge water, NJ). The 3 mixtures used in the animal acceptability evaluation were: (1) a 1:3:5 ratio of binder:diet:water using cassava starch; (2) a 1:5:7 mixture using hydroxypropyl methylcellulose; and (3) a 1:5:7 mixture using carboxymethylcellulose (Table 1).

Evaluation of animal acceptability

An acceptability trial was conducted to test the boluses using 12 5-month-old B6D2F1 mice of conventional microbiological status. Animals were allocated to one of 3 groups, 2 males and 2 females in each group. All animals were acquired from the National Center for Toxicological Research, Jefferson, AR, Breeding Facility. The boluses contained 1 g of ground (20 mesh) NIH-31 diet hand-mixed with binder and water in the ratios determined in the previous stage of testing. Environmental conditions of the animal rooms were $23 \pm 1{\cdot}5$ °C temperature, 50 ± 9% relative humidity, automatic 12:12 light:dark cycle (lights on at 0600 h), and HEPA filtered air with 10–15 exchanges/h. The animals were individually housed; food and water was available *ad libitum* before and after the testing. The mice were fasted for 24 h immediately prior to testing to promote complete and rapid consumption of the bolus. Polycarbonate shoebox cages were modified for the test by gluing a small, preweighed beaker containing the bolus to the cage wall one-inch from the floor to prevent contamination of the bolus with faeces or urine. Bedding was removed from the cages during the 1 h of exposure to the bolus in order to accurately observe the animals' acceptance. The animals were continuously observed to determine quantity consumed and wastage or spillage of the bolus.

Evaluation of dosing efficiency

Twelve male and 12 female 5-month-old B6D2F1 mice were used in this section of the study. Environmental conditions were the same as in the evaluation of animal acceptability. The animals were allocated to 2 replicate groups of

6 males and 6 females. While one group was being tested, the other group was housed in polycarbonate shoebox cages with *ad libitum* food and water provided. During collection intervals, animals were housed in polycarbonate metabolism cages (Maryland Plastics Inc., Federalsburg, MD) and acclimatized for 3 days prior to the trial.

The test was conducted in consecutive treatment order with each animal receiving all treatments with intervening intervals in which the effects of the previous treatment were determined by urine assay as described below. Testing of the high-moisture bolus, water bottles, and gavage was performed in consecutive 4-day periods. Urine was collected on the first day (Day 0) of each period to establish baseline D-xylose excretion values and to confirm that D-xylose excretion had returned to baseline values before beginning another treatment. The dose was provided after baseline urine samples were collected and analysed. Urine was collected for 3 consecutive days following dosing to ensure complete recovery of the D-xylose. To prevent bacterial growth in the urine samples, 0·2 ml of a 10% thimerosal solution (Aldrich Chemical Co., Milwaukee, WI) was added to each sample prior to analysis (J Knowles, personal communication).

The high-moisture diet bolus, 63% dry matter, contained 1·0 g ground (20 mesh) NIH-31 rodent meal, 0·1 g Methocel©hydroxypropyl methylcellulose as the binder, and 1·6 ml of a 6·25 g D-xylose/100 ml water solution to provide 0·1 g D-xylose/dose. The boluses were mixed by hand and provided to the mice for 24 h. Consumption was measured as change in weight of the beaker and contents. No spillage or wastage was noted. Water was available *ad libitum* throughout this treatment. The NIH-31 pelleted diet was returned following bolus consumption.

The water bottle dose consisted of 1·0 g D-xylose in 100 ml water placed in the water bottles for a 24-h period. Intake was represented as a change in weight of the water bottle and contents. The concentration of D-xylose consumed was then calculated. Fresh water was returned after the treatment. NIH-31 pelleted diet was available *ad libitum* throughout this treatment.

The gavage dose was 0·5 ml of a 5·0 g D-xylose/50 ml water solution (0·05 g D-xylose/dose) administered via syringe and blunt-ended gavage needle. Water and NIH-31 pelleted diet were available *ad libitum* throughout this treatment.

Sample analysis

The urine samples were analysed using a modified colorimetric method of Eberts (Eberts *et al.*, 1979) in which benzoic acid was omitted from the assay. Five standards containing 0·5, 1·0, 2·5, 5·0 and 10·0 mmol/l of D-xylose were prepared to establish daily calibration curves. Consistent linear calibration curves were established using standards prepared by the modified procedure. Samples were prepared in 35 ml screw-top tubes with Teflon©-lined caps and incubated in a boiling water bath. When the D-xylose content of a sample was below detection limits, the assay was repeated using 2 or 4 times the urine concentration to achieve a measurable D-xylose concentration. Results were adjusted for the increased urine concentrations. All samples were run in triplicate and averaged, samples were reanalysed if the variance exceeded 10%.

Statistical analysis

The main effects of sex and treatment upon D-xylose recovery between replications were the factors considered. Statistical analyses were performed by using analysis of variance obtained from the General Linear Models procedure of Statistical Analysis Systems (SAS, 1982) using least squares calculation of treatment means and F-protected comparisons. Tukey's test was also employed to verify multiple means comparisons.

Results

Evaluation of animal acceptability

The criteria for evaluating the boluses were: (1) consumption by the mice and, (2) cohesive integrity of the bolus while the mice were feeding. The bolus containing cassava starch was consumed

by half of the mice and showed high cohesive integrity. The bolus containing the carboxymethylcellulose was consumed by all of the mice but showed poor cohesive integrity. The bolus containing hydroxypropyl methylcellulose was consumed by 3 of the 4 mice and showed high cohesive integrity. Based on these results, hydroxypropyl methylcellulose was selected for use in the bolus preparation for further testing. Hydroxypropyl methylcellulose has been proven safe for long- and short-term use with rodents (WHO, 1974).

Evaluation of dosing efficiency

The recovery efficiency of each dosing treatment was calculated as per cent of the original D-xylose dose which was recovered in the urine samples. The average D-xylose dose received by each animal was $0{\cdot}091 \pm 0{\cdot}010$ g for animals consuming the high-moisture bolus, $0{\cdot}042 \pm 0{\cdot}012$ g for animals receiving water bottles, and 0·05 g for gavaged animals (no variance). Two male mice died during the study. During the first replication, one mouse died from unknown causes after the final water bottle treatment period. During the second replication, one mouse died from accidental intubation of the lungs during the gavage procedure. Statistically, the losses only reduced the observations for the gavage comparison where $n = 11$ for the males.

As there was no statistical difference due to replication, results were pooled for comparison of per cent D-xylose recovery by sex and treatment (Table 2). Females had significantly higher D-xylose recoveries than males for all methods tested. Recovery among females for the high-moisture bolus, gavage and water bottle treatments differed $P<0{\cdot}10$, $P<0{\cdot}01$ and $P<0{\cdot}05$, respectively, as compared with male recoveries. Among females, significantly higher ($P<0{\cdot}05$) total D-xylose recoveries were found for the water bottle and high-moisture bolus methods, 59·6 and 54·1%, respectively, as compared with gavage, 47·1%. Among males, significantly higher ($P<0{\cdot}05$) total D-xylose recoveries were found for the water bottle and high-moisture bolus methods, 52·5 and 46·0%, respectively, as compared with gavage, 35·5%. The trends for both sexes showed a similar hierarchy due to treatment (water bottles > high-moisture bolus > gavage).

Table 2. Per cent recovery of total xylose dose from high-moisture bolus, gavage and water bottle treatments among 5-month-old B6D2F1 mice

	Treatment, % Recovery			
Sex	*High-moisture bolus*	*Gavage*	*Water bottle*	*SEM*
Female	54·1[a]	47·1[b]	59·6[a]	2·30
Male	46·0[a]	35·5[b]	52·5[a]	2·87

[a,b]Means in a row with different superscripts differ ($P<0{\cdot}05$).

Table 3. Per cent daily xylose recovery from high-moisture bolus, gavage and water bottle treatments among 5-month-old B6D2F1 mice

		Daily per cent recovery		
Sex	*Treatment*	*1*	*2*	*3*
Female	High-moisture bolus	51·8[a]	2·2	0·16
	Gavage	44·9[b]	1·3	0·97
	Water bottle	57·9[a]	1·9	−0·18
	SEM	2·09	1·04	0·634
Male	High-moisture bolus	40·2[a,b]	6·1	−0·30
	Gavage	32·5[b]	2·1	0·87
	Water bottle	44·3[a]	6·3	1·89
	SEM	4·00	2·52	0·683

[a,b]Means in a column with different superscripts differ ($P<0{\cdot}05$).

The D-xylose recoveries for the first day (Table 3) following each treatment showed significantly ($P<0{\cdot}05$) greater recoveries for the high-moisture bolus and water bottle treatments as compared to gavage dose recovery, 51·8 and 57·9% vs 44·9%, respectively. For males, water bottle and high-moisture bolus treated animals demonstrated significantly ($P<0{\cdot}05$) more efficient first day D-xylose recoveries than gavage, 44·3 and 40·2% vs 32·5%, respectively. Essentially, all D-xylose recovery was complete by 2 days post-treatment.

Discussion

The 3 methods for oral administration of test compound used in this study have various

advantages and disadvantages that determine their suitability for a study. When the comparative efficiency of gavage, water bottles, and a novel high-moisture bolus in delivering a known quantity of D-xylose to male and female B6D2F1 mice was analysed, water bottle delivery demonstrated the highest D-xylose dosing efficiency and recovery of the 3 methods. However, the efficiency of the high-moisture bolus delivery was not statistically different from that of the water bottles.

For each dosing method tested, there are associated disadvantages. For the high-moisture bolus, the bolus must be given separately from normal feeding regimens, requires added labour, and a pre-fast interval to ensure complete consumption. For the water bottles, dose loss due to spillage is a commonly cited disadvantage (Lang *et al.*, 1984). In this study, one animal died after accidental intubation of the lungs following gavage.

It is not known whether the binder used in the high-moisture bolus had any inhibitory effect on D-xylose absorption; however, gel-forming gums, including carboxymethylcellulose, have been shown to reduce D-glucose transport in the rat jejunum (Johnson & Gee, 1981). If D-xylose and D-glucose share a common transport pathway, as suggested by some researchers (Ohkohchi & Himuki, 1984), the inhibition of transport by the hydroxypropyl methylcellulose gel used could have reduced the efficiency of D-xylose absorption from the high-moisture bolus. Based on D-xylose absorption studies using rats, the minor differences in concentrations of D-xylose provided by the gavage and water bottle solutions (0·05 and 0·042 g, respectively) should not have changed the proportion of D-xylose absorbed (Stradley *et al.*, 1986).

The greatest recovery for D-xylose observed in this study was an average 56% following water bottle administration (Table 2). Earlier researchers (Segal & Foley, 1959) report that labelled D-xylose infusion in man resulted in an average 44% urinary recovery of the total dose. These researchers proposed that the pentose sugars may be converted, in part, to glucose or may enter glycogenesis via the pentose phosphate pathway. This latter conversion had been reported to occur in the intact mouse (Hiatt, 1957).

A significant sex difference was seen in all treatments in which D-xylose recovery among females was consistently higher than among males. A study investigating sex differences in human D-xylose excretion found a tendency among females to excrete D-xylose more efficiently than males after an intravenous dose (Kendall & Nutter, 1970). Similar research has not been previously conducted for mice.

As gavage dosing is widely used as a method of chemical administration, the results reported here are of particular interest. The comparison of other compounds among the 3 dosing methods is warranted to provide more information about the recovery efficiencies of the methods studied.

Xylose is the chief pentose that is actively absorbed from the gut (Roehrig, 1984). Absorption of the total D-xylose dose may have been inhibited by the saturation of the active transport system. Although D-xylose was selected for its previously reported stability, perhaps this pentose sugar is metabolized as previously suggested (Hiatt, 1957).

The summarized data show that both water bottles and the high-moisture bolus are comparable in delivering a known quantity of D-xylose to mice. Water bottles showed a consistently higher, but not significantly different, efficiency than the high-moisture bolus, but the method is limited to water-soluble compounds. The high-moisture bolus is more versatile with regard to compound solubility.

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